family of DNA transposons. Although it is an important tool in genetic applications and has been adapted for human gene therapy, its molecular mechanism remains obscure. Here, we use a combination of NMR and CD spectroscopy and molecular modeling to show that the primary DNA recognition domain of the Sleeping Beauty transposase, the PAI subdomain, is well folded at low temperatures, but the presence of unfolded conformation gradually increases at temperatures above 15°C. Furthermore, we show that only the folded conformation of the PAI subdomain binds to the transposon DNA, suggesting that the choice of temperature may be important for the optimal transposase activity. To gain insight into the mechanism of hyperactive mutations K14R and K33A located in the PAI subdomain of SB transposase, we determine the effects of mutations on its folding and DNA-binding properties. Overall, the results provide a molecular-level insight into the DNA recognition by the PAI subdomain of SB transposase.

247-Pos Board B27
CPSF30, a Novel Non-Classical Zinc Finger Protein that Utilizes Iron & Zinc Coordination for RNA Recognition
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Zinc finger (ZF) proteins are eukaryotic proteins that are involved in transcriptional and translational regulation. ZFs utilize cysteine and histidine residues to coordinate zinc ions to fold and function properly. There are fourteen different classes of zinc finger proteins, which are classified based upon the ligands involved in zinc coordination, the ZF domain folds, and the proteins' biological targets. Cleavage and Polyadenylation Specificity Factor 30 (CPSF30) is a 'non-classical' ZF protein that contains multiple Cys3His zinc binding domains and is involved in pre-mRNA regulation. CPSF30 contains 5 Cys3His domains and a Cys2HisCys zinc knuckle domain. A construct of CPSF30 construct containing only CPSF30's five Cys3His domains, denoted as CPSF305FE was prepared. This protein is predicted to have five zinc ions; however, UV-visible and ICP-MS analysis of the isolated construct revealed that CPSF305FE contains iron in addition to zinc in a 1:4:1 Fe:Zn:CPSF305FE stoichiometetry. XAS analysis identified the iron site as a 2Fe-2S cluster. CPSF30 is predicted to recognize AU-rich RNA sequences. To test this hypothesis, RNA binding studies using both electrophoretic mobility shift assays (EMSA) and fluorescence anisotropy (FA) of CPSF305FE with α-synuclein pre-mRNA (which contains an AU-rich sequence) along with a series of mutated RNA sequences were performed. These studies revealed that CPSF30 binds to α-synuclein pre-RNA with nanomolar affinity and requires the AU-rich sequence for binding. The binding interaction was best fit to a cooperative binding model, suggesting that the CPF30/RNA binding event is cooperative.

Protein Folding and Chaperones

248-Pos Board B28
Transition Path Times in Protein Folding Studied by Structure-Based Simulation
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In trajectories of protein folding and unfolding processes, the transition path that connects denatured and native states is the most interesting part. Recently, single-molecule FRET experiments together with a statistical inference theory successfully identified the transition path times for folding of some small proteins. Yet, its underlying physics is poorly understood. Here we conducted a comprehensive survey of transition path times for 29 small-to-medium two-state-folding proteins using structure-based coarse-grained molecular dynamics simulations. Using the multi-canonical ensemble method, we first identified folding transition temperature accurately. At the transition temperature, we then performed relatively long simulations observing reversible folding and unfolding events, from which we identified and analyzed the transition path times. The distribution of transition path time for each protein can be explained as free diffusion of particle in a reaction coordinate. We sought what reaction coordinates correlate with the transition path time. Among tested coordinates, we found that the average transition path time is most strongly correlated to the difference in the numbers of native contacts, i.e., contact energies, between native and denatured states. These results imply that the transition path time series can be approximated as the nearly free diffusion in the reaction coordinate of native contacts.

249-Pos Board B29
Folding Rates from Thermodynamics Simulations: Apoazurin as an Example
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In this work we employed the approach of the Mean First Passage Time (MFPT) to calculate protein-folding rates from the thermodynamic simulations according to the Energy Landscape Theory developed by Bryngelson and Wolynes. The folding rates from MPFT depend on a single reaction coordinate on the energy landscape. Here we applied this technique with the aid of coarse-grained molecular simulations for the computation of folding rates of protein Apoazurin. Experimentally, apoazurin folds in a two-state manner that allows for the use of a structure-based model attributable to a smooth funnel-like energy landscape. Based on the fraction of native contacts as the choice of a single reaction coordinates, we computed the folding rates of Apoazurin at several cellular conditions that perturb the population shift of the energy landsape.

250-Pos Board B30
Barrierless Transition Identified during Folding of Barstar by using Time-Resolved FRET from 5-Flurotryptophan
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Despite a large volume of theoretical support for barrierless (downhill) folding of proteins, the extent of experimental observation is rather limited. This limitation is even more striking when we address the kinetic process of folding rather than equilibrium titrations of folding-unfolding transition. In this work we use the fluorescence of a single 5-Fluorotryptophan (F-Trp) in barstar as the energy donor and study the evolution of intramolecular distances and distance distributions during the kinetics of folding. Enhanced homogeneity of fluorescence decay kinetics of F-Trp when compared to that of Trp enables unequivocal interpretation of distribution of fluorescence lifetimes obtained from the Maximum Entropy Method (MEM) as arising from folded and unfolded conformations of the protein. Of the two intramolecular distances monitored during the folding process, W53-C82 distance showed continuous decrease while W53-C40 distance behaved in a two-state manner. Implication of these observations to the folding process is discussed.

251-Pos Board B31
Development of the Line Confocal System for the Single Molecule Tracking of Fast Folding Dynamics of Proteins
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Single-molecule fluorescence spectroscopy is a powerful method for the investigation of protein dynamics. However, the time resolution of single-molecule fluorescence spectroscopy is usually limited to a few milliseconds. To improve the time resolution, we developed the line-confocal microscope combined with microfluidic chip. By using the device, we could trace the time evolution of FRET efficiency from single molecules with the time resolution of about 100 μs for the observation time of 5 ms. As the first example of the developed method, we investigated the equilibrium unfolding transitions of two mutants of the B domain of protein A (BdpA) doubly labeled with donor and acceptor fluorophores. In the case for the mutant with a shorter donor-acceptor distance, the FRET traces revealed a conformational heterogeneity in the unfolded state. In the case for the mutant with a longer donor-acceptor distance, the presence of a minor conformation in the native state was detected. These results demonstrate that the developed method can reveal the complexity in the apparent two-state folding of BdpA. To track the conformational fluctuations of denatured proteins and the fast protein folding transition, we are improving the system. To obtain the better time resolution without sacrificing the observation time, we built a new system based on hybrid photo detectors (HPD). HPD is a photon-counting detector which achieves a high sensitivity and a large effective area. By introducing HPD to the line-confocal microscope, we could obtain the single-molecule FRET traces with the time resolution of 10 μs and the observation time of more than 10 ms. The traces of BdpA obtained by using the new system exhibited